ANALYSIS

17(47), 2020

ISSN 2319–7757 EISSN 2319–7765

Indian Journal of Engineering

Biokinetics of Crude Oil Remediation using Dogoyaro (*Azadirachta indica*) Stem.

Promise NU[1], **Ukpaka CP**[2], **Puyate YT**[3]

[1]Postgraduate Researcher Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Nigeria
[2]Professor Chemical/Petrochemical Engineering Rivers State University Port Harcourt. E-mail: chukwuemeka24@yahoo.com
[3]Professor Chemical/Petrochemical Engineering Rivers State University Port Harcourt, Nigeria

Article History
Received: 10 March 2020
Accepted: 16 April 2020
Published: April 2020

Citation
Promise NU, Ukpaka CP, Puyate YT. Biokinetics of Crude Oil Remediation using Dogoyaro (*Azadirachta indica*) Stem. *Indian Journal of Engineering*, 2020, 17(47), 250-260

ABSTRACT

The study of a biokinetic model development for bioremediation of 1kg of crude oil contaminated soil inoculated with crushed sun-dried and room-dried *Azadirachta indica stem* was conducted. First, physico-chemical analysis was carried out on the samples used in the experiment. The experiment was carried out ex-situ in twenty-six batch reactors, two which were the control. The other twenty-four reactors containing 1kg each of two types of soil: sandy in twelve reactors and loamy in another twelve were polluted with 100ml of Bonny light crude oil and inoculated with two samples of crushed *Azadirachta indica* stem. The quantities of Azadirachta indica stem were varied from 50g to 100g at 10g difference. The set-up was observed for 35 days as GC-MS analysis was done on the soil samples on weekly basis to determine crude oil depletion in each reactor. It was observed that contaminated soils treated with 100g blended room-dried *Azadirachta indica stems* had the highest TPH decline from 48508.92ppm to 3707.78ppm for loamy soils (LRD) and 35818.69ppm to 1003.18ppm for sandy soils (SRD). Based on this observation, a predictive model was developed for either soil samples using both Analytical methods and numerical methods that predicted with high precision, the TPH content of the soil at any given time.TPH at 21 days was calculated to be 9085.47mg/kg which is similar to that obtained from experiment observed to be 9085.48mg/kg. The highest error was observed in day 7 where calculated TPH was 23477.06mg/kg while the experimental value was 26865.7mg/kg. For LRD, TPH at 35 days was calculated to be 3707.81mg/kg which

is similar to that obtained from experiment observed to be 3707.78mg/kg. The highest error was observed in day 7 where calculated TPH was 35707.07mg/kg while the experimental value was 37851.62mg/kg.

Key words: Biokinetics, crude oil, remediation, Dogoyaro, *Azadirachta Indica*, stem.

1. INTRODUCTION

There is no gain saying that the upstream, midstream and downstream operations of the oil sector is the paramount cause of environmental pollution in Nigeria. After oil discovery in 15th January 1956, there have been numerous oil-spill cases: between 1976 and 1996 alone, a total of 4647 incidents resulted in the spilling of about 2,369,470 barrels of oil into the environment, of which an estimated 1,820,410.5 barrels (77%) were not recovered [1]. This amount of crude oil left unmitigated in the soil could pose very precarious threats to the environment and must be taken care of immediately in order to preserve aquatic or plant life and soil nutrients [2-3].

Paradigms for treating crude oil contaminated soils include incineration, soil vapor extraction, solidification/stabilization, soil washing, bioremediation etc. Some of these methods have their disadvantages because in the aftermath of the treatment, toxins noxious to plant and animal life are released into the soil [6-8]. Aside this, these methods are relatively expensive hence the need for cheaper alternatives, cost effective and very effective means of control [4-5]. In recent times, bioremediation has become one of the most propitious technologies with increasing demand for remediating crude oil contaminated soils because contaminants can be removed using innocuous and soil-friendly microbial colonies [6].

Numerous studies have established bioremediation as a cheap, eco-friendly and effective way of amending soils contaminated with crude oil. Studied conducted on plant extract of various species and the use of two species of bitter-leaf; *Vernonia Galamensis* and *Vernonia Amygdalina*, in the bioremediation of crude oil contaminated soils reveals their significant on crude oil remediation [7-8]. The leaves of each species were applied to the contaminated soil either room dried, sun dried or wet and blended [9]. The microbes (*Pseudomonas Aeruginosa, Staphylococcus Aureus* and *Eureschia Coli*) and phytochemicals in the leaf extracts were the agents responsible for the degradation of the metals as well as the hydrocarbon contents in the soil [10-12]. Results showed that the use of forty-gram of both species of *Vernonia* extracts reduced more than fifty-percent of the contaminants concentration in the soil, after forty days of investigation [13-15]. Therefore, the extracts of both species of *Vernonia* were effective as agents of bioremediation in a crude oil contaminated soil. However, studies conducted by various research groups recommended that, for the remediating effect of the either of the extract of both *Vernonia*species (depending on which is available) to be maximized, it should be applied to the polluted soil wet and blended [16-20]. This is because the microorganisms present in the leaves to carry out the bioremediation are still active [21-24]. The result from this study showed that wet blended *Vernonia* extracts remediated more than fifty percent of the initial crude oil content of the contaminated soil [25].

Investigation on the possibility of enhancing crude oil bioremediation by supplementing soil with cost-effective organic materials derived from two widespread trees grown in Kuwait, *Conocapus* and *Tamarix* [25-26].The leaves of both plants were sterilized and blended and phytochemical analysis was carried out on the soil. Amendments in soils increased the soil's microbiota count by up to Ninety-Eight percent and enhanced their activity by up to Ninety-Five percent [27-30].

Studieson the effectiveness of grounded neem tree leaves in crude oil bioremediation process. Results showed that the leaves of *Azadirachta indica* was able to support and promote the growth of hydrocarbon degraders (Pseudomonas species) indicating that they play complementary role in bioremediation [31]. The biostimulation potential of *Azadirachta indica* leaves in increasing the microbial population in crude oil contaminated vessel may be due to the nitrogen and phosphorus content of the stem [32]. Increase remegiation of polluted site was observed when using NPK fertilizer, urea fertilizer and poultry droppings effectively stimulated the growth of bacteria into utilization of crude oil [33].The research carried out established that the leaves of *Azadirachta indica* is very effective in bioremediation of crude oil contaminated soil by stimulating the growth of crude oil utilizing endophytes [34].

Obviously, in recent time enormous research had been carried out on bioremediation. However, not much work has been done in the mathematical modelling of the process involved in it. This study adopted a Monod-based-mathematical model to describe the bioremediation process of crude oil contaminated soils with *Azadirachta indica stem* as remedant [35-36].

Owing to the discrepancies in the physico-chemical properties of different soils and the difference in chemical constituents of different crude oil samples in different environments, previous models may not be adequate enough to describe the treatment of oil spill in the Niger Delta 37-38]. For Instance, models developed by some research groups, describes crude oil bioremediation in arid

Tehran may not be useful in describing crude oil remediation in Niger-Delta [39].This study aimed at designing a simple andrealistic model that describes the treatment of crude oil contaminated soils.

2. MATERIALS AND METHODS

Materials and Equipment Used for the Experiment

The soil samples and the *Azadirachta indica* leaves were obtained from a farm within Rivers State University vicinity.

Sample Preparation and Experimental Setup

Twenty-six (26) reactors were used in the experiment of which 2 were control experiment containing crude oil contaminated soils without bioremediant. The other 24 reactors contained crude oil contaminated soils thoroughly mixed with varying quantities of either sun-dried or room-dried blended *Azadirachta indica stem*. The crude oil sample obtained was investigated for Total Petroleum Hydrocarbon content. The sandy and loamy soils were weighed 1kg into thirteen places for each type of soil. Some of the *Azadirachta indica stem* were sun-dried for 7 days and the others were room-dried for 14 days. Both the sun-dried and room-dried leaves were thoroughly blended.The weighed soil samples were contained in 26 reactors, 1kg of soil to 1 reactor. Each 1kg of soil sample was then contaminated with 100ml of crude by thorough mixing it with the soil. The blended sun-dried stems were weighed ranging from 50g to 100g with one replicate for each measure. The room-dried stems were weighed ranging from 50g to 100g with one replicate for each measure also. This summed up to 24 weighed blended stem samples. The weighed stemsamples were systematically applied on 24 of the contaminated soil samples. Below is a schematic representation of the experimental set up:

Table 1: Schematic Representation of the Experimental Set-Up.

50g		60g		70g		80g		90g		100g	
Sandy	Loamy	sandy	loamy	Sandy	Loamy	sandy	loamy	sandy	Loamy	sandy	loamy
SSD	LSD	SSD	LSD	SSD	LSD	SSD	LSD	SSD	LSD	SSD	LSD
SRD	LRD	SRD	LRD	SRD	LRD	SRD	LRD	SRD	LRD	SRD	LRD

SSD represents the reactor containing sandy soil contaminated with crude oil and inoculated with blended sun-dried *Azadirachta indica stem*. SRD is the same with SSD except that blended room-dried *Azadirachta indica stems* were used instead of the sun-dried. To each type of soil contaminated with crude oil, quantities of either sun-dried or room-dried blended *Azadirachta indica stem* ranging from 50g to 100g were added as illustrated in table 1. Each combination was thoroughly mixed to ensure uniformity of the components.

Before inoculation, the contaminated soils were analyzed for initial conditions like Total Hydrocarbon Content, initial microbial activity, conductivity, pH etc. Subsequently, the 24 experimental samples were analyzed every week for a period of 8 weeks. Within this period, 10ml of water was added to each of the 24 inoculated soil samples to aid easy movement of microbes.

Gas Chromatography Procedure

The soil sample was poured into a 1 liter separation funnel. 50mL of methylene chloride was added to the sample bottle seal and shook for 30 seconds to rinse the inner surface. The solvent was transferred to the separation funnel and then sample was extracted by shaking the funnel for 2 minutes with periodic venting to release excess pressure.

The organic layer was allowed to separate from the water phase for a minimum of 10 minutes and then the methylene chloride was extracted in a 250mL flask. A second 60mL volume of methylene chloride was added to the sample bottle. The separation funnel and the column was rinsed with 20mL of the solvent into the extract. The extraction procedure was repeated a second time and combined with the other extract in an Erlenmeyer flask. The third extraction was performed in the same way. The combined extracts were poured through a drying column containing packed cotton wool, anhydrous sodium sulphate and silica. The extract was collected in the vial and concentrated by boiling it down with nitrogen gas to 1.0mL. The remaining extract was mixed with 1.0mL of the solvent and 1.0µL was injected into the flame ionization detector gas chromatograph for the TPH analysis.

Developing the Biokinetic Models

The possible biochemical reaction to be observed as a result of micro-organism in a crude oil contaminated site is as shown below:

Product oil + O_2 + Nutrients ⟶ CO_2 + H_2O + New Biomass + Heat released

From the equation shown above, the following mathematical models can be developed to illustrate the kinetics of production of biogenic gases produced in a reservoir for biodegradation of the soil.

The Microbial Kinetics

When a microorganism undergoes the biochemical reactions for dynamic studies, the general conservation for a steady state must be modified to give the following unsteady state mass balance.

$$\begin{bmatrix} rate\,of\,oil\,flow \\ into\,the \\ system \end{bmatrix} - \begin{bmatrix} rate\,of\,oil\,flow \\ out\,of\,the \\ system \end{bmatrix} - \begin{bmatrix} rate\,of\,oil \\ consumption\,by\,microbes \end{bmatrix} = \begin{bmatrix} rate\,of\,oil\,accumulation \\ in\,the\,system \end{bmatrix} \quad (1)$$

For a batch system, the following assumptions hold true:

1. The rate of oil flow into the system = 0
2. Rate of oil flow out of the system = 0

Hence equation (1) can be reduced to:

$$-\begin{bmatrix} rate\,of\,oil \\ consumption\,by\,microbes \end{bmatrix} = \begin{bmatrix} rate\,of\,oil\,accumulation \\ in\,the\,system \end{bmatrix} \quad (2)$$

$$-\frac{dS}{dt} = \frac{1}{Y_{\frac{X}{S}}} \times \frac{dX_t}{dt} \quad (3)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{\frac{X}{S}}} \times \frac{dX_t}{dt} \quad (4)$$

But;

$$\frac{dX}{dt} = \mu X_t \quad (5)$$

Hence equation (4) becomes;

$$\frac{dS}{dt} = -\frac{\mu X_t}{Y_{\frac{X}{S}}} \quad (6)$$

Monod's equation states that;

$$\mu = \frac{\mu_m S_t}{K_m + S_t} \quad (7)$$

Equation (6) can now be rewritten as;

$$\frac{ds}{dt} = -\frac{\mu_m S_t X_t}{Y_{\frac{X}{S}}(K_m + S_t)} \quad (8)$$

Where, μ is the specific growth rate of microbes in the system per week, S_t is the quantity of crude oil available at time t, X_t is the microbial count at time t, $Y_{X/S}$ is the cell yield constant, $\frac{dS}{dt}$ is the rate of depletion of the oil, $\frac{dX}{dt}$ is rate at which the microbes proliferates, μ_m is the maximum specific growth rate of the microbes in the system and K_m is the half saturation constant

Determining the values of μ_m, K_m and $Y_{x/s}$

To determine μ_m and K_m, a graph of 1/μ against 1/S was plotted. This implies that equation (7) had to be converted to an inverted form to achieve a straight line graph, i.e.

$$\frac{1}{\mu} = \frac{K_m}{\mu_m}\frac{1}{S_t} + \frac{1}{\mu_m} \quad (9)$$

Where,

$\frac{K_m}{\mu_m}$is the slope of the graph and $\frac{1}{\mu_m}$ is the intercept

First, the values of μ were calculated using equation (10) below:

$$\mu = ln\left(\frac{X_t}{X_o}\right) \times \frac{1}{t} \quad (10)$$

Where, X_o and $X_t$are the initial microbial count and microbial count at time t respectively.

$Y_{x/s}$ was determined using equation (11) below:

$$Y_{x/s} = \frac{X_t - X_o}{S_o - S_t} \quad (11)$$

Make X_t the subject of formula of equation (11), we have

$$X_t = Y_{x/s}(S_0 - S_t) + X_0 \quad (12)$$

Substituting equation (12) into equation (8), we have

$$\frac{ds}{dt} = \frac{\mu_m Y_{x/s} S_t^2 - \mu_m S_0 Y_{x/s} S_t - \mu_m X_0 S_t}{Y_{x/s} K_m + Y_{x/s} S_t} \quad (13)$$

Evaluation of Equation (13)

Equation (13) was solved using analytical methods to yield equation (14) below:

$$a_1 log_e(S - a_2) - a_3 log_e S - a_4 - t = 0 \quad (14)$$

Where, a_1, a_2, a_3 and a_4 are arbitrary constants with values 30.9397599, 1371.64227752, 49.058803, -191.2084567 respectively for SRD and 19.6768957, 4567.1603183, 43.7196450 and -261.3550175 respectively for LRD. The value constants were obtained by using the collocation method and Newton-Raphson's method. S is the Total Petroleum Hydrocarbon content.

3. RESULTS AND DISCUSSION

Data Analysis and Results

This research work deals with the experimental results obtained from tests carried out, which was used in the computation to determine the functional parameters and coefficients. These results will lead to computations on the determination of maximum specific rate, specific rate and equilibrium constant. Results and data are represented on tables and figures. The figures will show similarity in the determination of maximum specific rate, specific rate and equilibrium constant for sandy soil and loamy soil each inoculated with 100g room-dried *Azadirachta indica stem*. Soil samples inoculated with 100g room-dried *Azadirachta indica* because they showed more promise than the rest set-up. The developed kinetics can be used to predict the entire biodegradation process of bonny light crude oil in Nigeria.

Microbial Growth Rate (μ) for SRD

μ was calculated using equation (10), we have.

For day 0 to day 7 where X_0 is 3.94×10^3cfu/g,X_t is 5.94×10^4cfu/g and t is 7, μ is 0.374cfu/g/week

For day 7 to day 14 where X_0 is 5.42×10^4cfu/g,X_t is 1.03×10^7cfu/g and t is 7, μ is 0.371cfu/g/week

For day 14 to day 21 where X_0 is 1.03×10^7cfu/g,X_t is 1.99×10^9cfu/g and t is 7, μ is 0.251cfu/g/week

For day 21 to day 28 where X_0 is 1.99×10^{9}cfu/g,X_t is 5.99×10^{10}cfu/g and t is 7, μ is 0.12cfu/g/week
For day 28 to day 35 where X_0 is 5.99×10^{10}cfu/g,X_t is 3.99×10^{11}cfu/g and t is 7, μ is 0.05cfu/g/week.

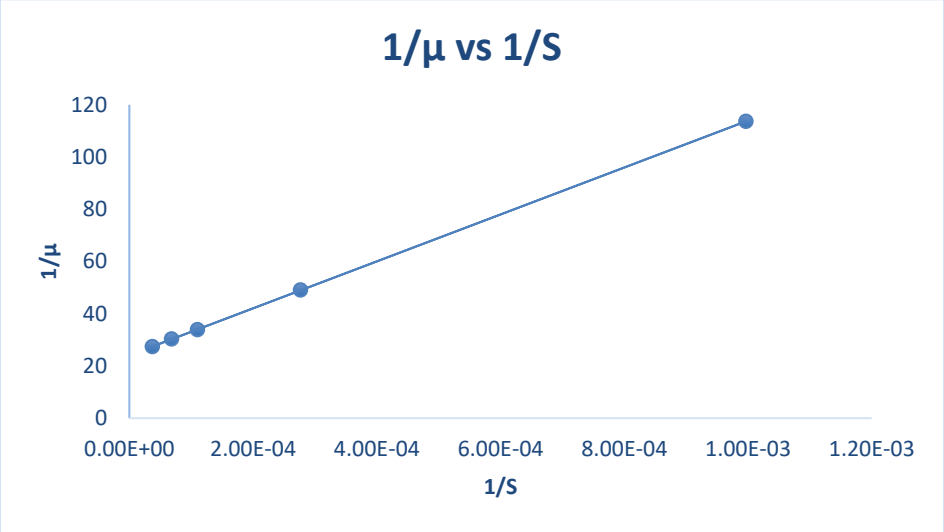

Fig 1: Line Weaver-Burk Plot for SRD

From Microsoft Excel, the equation of the plot in Figure 1 was found to be:

$$\frac{1}{\mu} = 89855.77\frac{1}{s} + 24.038 \qquad (15)$$

Comparing equation (15) to equation (9), we haveμ_m calculated to be 0.0416cfu/g/week and $K_m$to be 37380mg/kg. Equation (11) was used to obtain the value of $Y_{x/s}$as$2.596 \times 10^{9} cfu/g$ for $X_t = 1.987 \times 10^{14}$, $X_0 = 8.113 \times 10^{13}$, S_0= 35818.69 and S_t = 1003.18. Having obtained the values of constantsμ_m, $K_m$and $Y_{x/s}$ equation 12 can be represented as;

$$\frac{ds}{dt} = \frac{1.079\times10^{8}S_t^2 - 3.868\times10^{12}S_t - 3.375\times10^{12}S_t}{9.703\times10^{12} + 2.596\times10^{9}S_t} \qquad (16)$$

Microbial Growth Rate (μ) for LRD

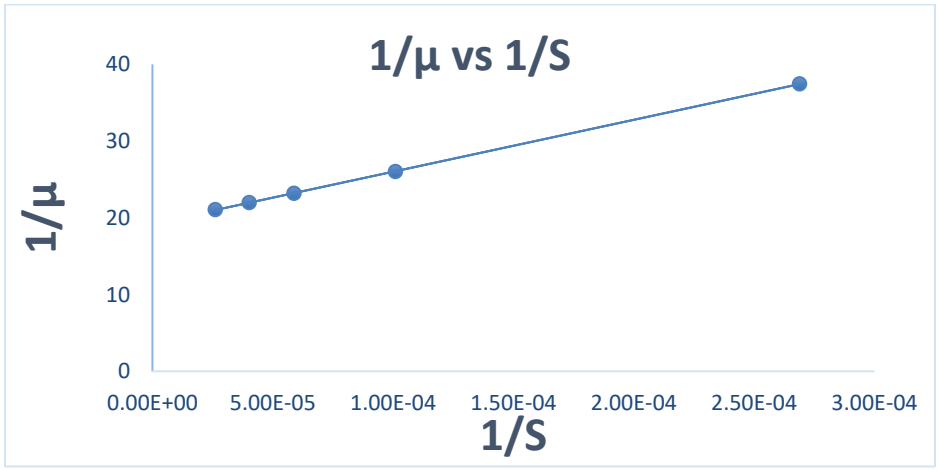

Fig 2: Line Weaver-Burk Plot for LRD

μ for LRD was calculated using equation (10), we have
For day 0 to day 7 where X_0 is 2.02×10^{10}cfu/g,X_t is 3.07×10^{10}cfu/g and t is 7, μ is 0.0475cfu/g/week
For day 7 to day 14 where X_0 is 3.07×10^{10}cfu/g,X_t is 4.22×10^{10}cfu/g and t is 7, μ is 0.0455cfu/g/week

For day 14 to day 21 where X_0 is 4.22×10^{10}cfu/g,X_t is 5.71×10^{10}cfu/g and t is 7, μ is 0.0431cfu/g/week
For day 21 to day 28 where X_0 is 5.71×10^{10}cfu/g,X_t is 7.471×10^{10}cfu/g and t is 7, μ is 0.0384cfu/g/week
For day 28 to day 35 where X_0 is 7.471×10^{10}cfu/g,X_t is 9.006×10^{10}cfu/g and t is 7, μ is 0.0267cfu/g/week

From Microsoft Excel, the equation of the plot in Figure 2 was found to be:

$$\frac{1}{\mu} = 67291\frac{1}{s} + 19.268 \quad (17)$$

Comparing equation 17 to equation 9,μ_m was calculated to be 0.0552/week and $K_m$to be 3713.8mg/kg. Equation (11) was used to obtain the value of $Y_{x/s}$as$4.67\times10^5 cfu/g$ for X_t = 9.006 × 10^{10}, X_0 = 2.202 × 10^{10}, S_0 = 48508.69 and S_t = 3707.78. Having obtained the values of constantsμ_m, $K_m$and $Y_{x/s}$ equation 12 can be represented as;

$$\frac{ds}{dt} = \frac{2.58\times10^4 S_t^2 - 9.23\times10^8 S_t - 1.214\times10^9 S_t}{1.73\times10^9 + 4.67\times10^5 S_t} \quad (18)$$

Comparison between the experimental TPH and Theoretical TPH

MATLAB was able to estimate the trend of TPH depletion with time. The calculated values of TPH for 35 days are presented in Table 2 below:

Table 2: Comparison between Theoretical Valuesand Experimental Values of SRD and LRD

Time (days)	TPH_{th} SRD (mg/kg)	TPH_{th}LRD (mg/kg)	TPH_{exp} SRD(mg/kg)	TPH_{exp} LRD (mg/kg)
0	35818.69	48508.92	35818.69	48508.92
7	23477.06	35707.70	26865.7	37851.09
14	15012.26	24890.51	14658.37	24890.09
21	9085.47	16999.28	9085.48	16998.99
28	3615.11	9875.66	3615.07	9897.95
35	1003.33	3708.81	1003.18	3707.78

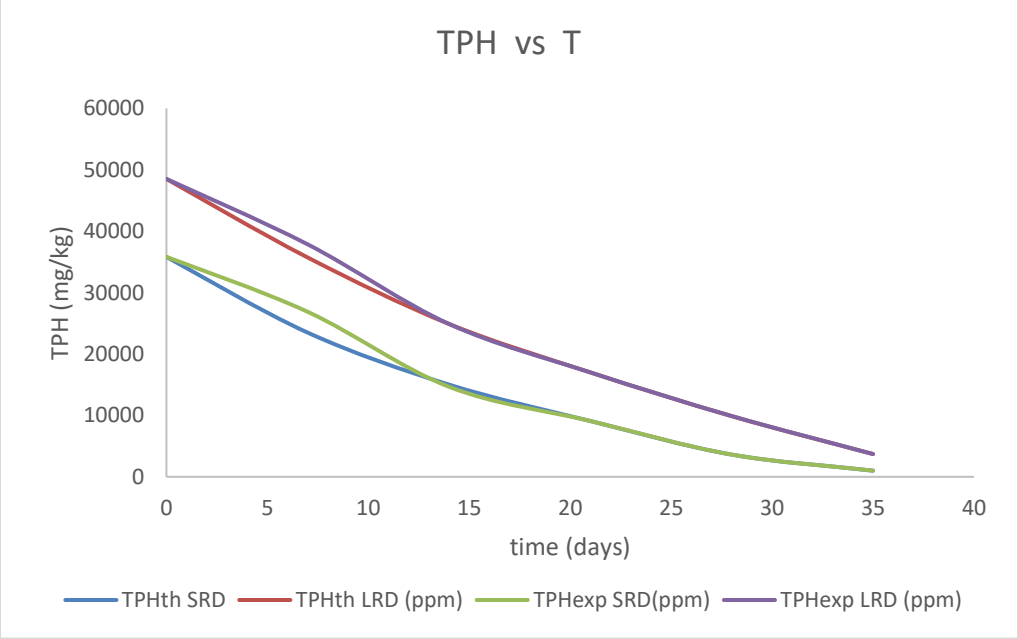

Fig.3: Comparison between Theoretical Values and Experimental Values of SRD and LRD

Using Analytical methods, equations (16) and (18) were solved to develop models that predict values of TPH of SRD and LRD respectively. Comparisons were made between theoretical values and experimental values using the percentage error equation:

$$P.E = \left|\frac{TPH_{exp} - TPH_{th}}{TPH_{exp}}\right| \times 100 (18)$$

Where, P.E is the percentage error, $TPH_{exp}$is the experimental value of TPH and $TPH_{th}$is the theoretical value of TPH estimated by the new model

The Table 3 below compares estimated values of TPH for SRD to their experimental equivalent.

Table 3: Comparison of Experimental Values of TPH for SRD to the Calculated Values

Time (days)	TPH_{exp} (mg/kg)	TPH_{th} (mg/kg)	ε_t (%)
0	35818.69	35818.69	0
7	26865.7	23477.06	12.6
14	14658.37	15012.26	2.4
21	9085.48	9085.47	0.004
28	3615.07	3615.11	0.016
35	1003.18	1003.33	0.014

The Table 4 below compares estimated values of TPH for LRD to their experimental equivalent.

Table 4: Comparison of Experimental Values of TPH for LRD to the Calculated Values

Time (days)	TPH_{exp} (mg/kg)	TPH_{cal} (mg/kg)	ε_t (%)
0	48508.92	48508.92	0
7	37851.62	35707.70	2.7
14	24890.09	24890.51	0.00156
21	16998.99	16999.28	0.00417
28	9897.95	9875.66	0.00293
35	3707.78	3708.81	0.000809

For SRD, TPH at 21 days was calculated to be 9085.47mg/kg which is similar to that gotten from experiment observed to be 9085.48mg/kg. The highest error was observed in day 7where calculated TPH was 23477.06mg/kg while the experimental value was 26865.7mg/kg.

For LRD, TPH at 35 days was calculated to be 3707.81mg/kg which is similar to that gotten from experiment observed to be 3707.78mg/kg. The highest error was observed in day 7 where calculated TPH was 35707.07mg/kg while the experimental value was 37851.62mg/kg.

Predicting TPH during Bioremediation using the Developed Model

Equation (14) in research work can be used to predict the TPH of the soil at any given time during bioremediation. The model was able to predict the TPH at Day 5, 10, 15, 30, 50, 55 and 60 picked arbitrarily. Table 5 and Figure 4 were used to illustrate the trend for both SRD and LRD.

Table 5: TPH Prediction for Arbitrary Days

Time (t)	TPH-SRD (mg/kg)	TPH-LRD (mg/kg)
5	27538.03	38531.61
10	19461.27	23644.18
15	14037.14	18023.21
30	1040.35	3845.07
50	202.19	620.41
55	187.20	589.11
60	171.79	522.65

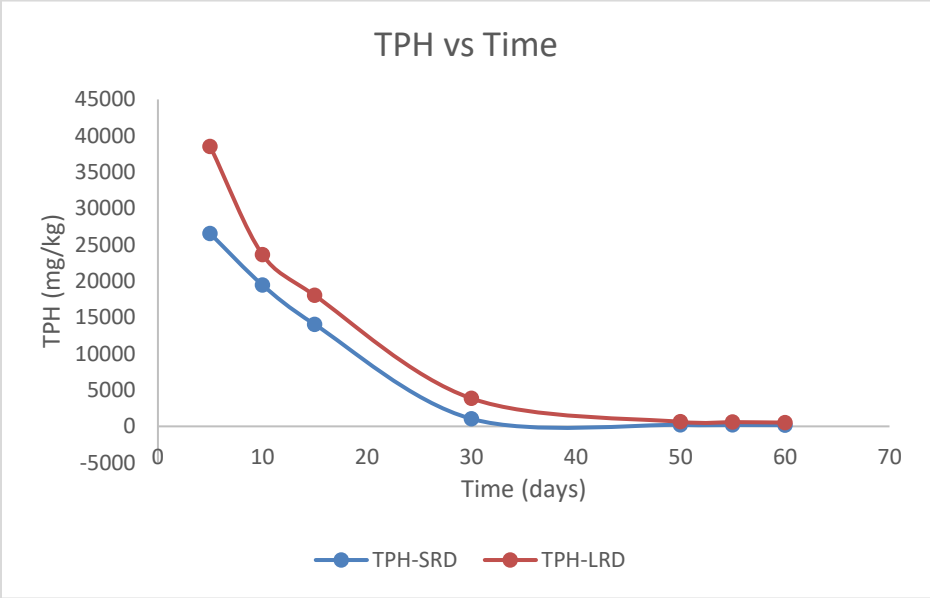

Fig. 4: A Graph of TPH Predictions for Arbitrary Days

The graph in Figure 4 established the accuracy of the developed predictive model. An attempt was made at interpolation using the predictive model, to find out TPH measures for arbitrary days within the period of experiment, thereby evaluating their accordance to the trend observed in the experiment. Sequel to day 7 of SRD whose TPH measure is 26865.7mg/kg from experiment, day 5 predicted by the model was seen to be 27538.03mg/kg indicating reasonable precedence. Also, between the theoretical TPH at day 5 and experimental TPH at day 7 for LRD, reasonable precedence was exhibited as the former has a TPH measure of 38531.61mg/kg and the later has a TPH measure of 37851.09mg/kg. Attempt was also made at extrapolation using the predictive model, to check the consistency between TPH for arbitrary days outside the range of days used in the experiment and the TPH measure for the last day of the experiment. The model predicted that at day 50, the TPH measure for SRD would decline to 202.16mg/kg which is a massive decline from the experimental 1003.18mg/kg at day 35. Also for LRD, the TPH measure predicted by the model would be 620.41mg/kg at day 50 which is a massive decline from the experimental 3707.78mg/kg at day 35. Hence, the model developed is reliable in predicting the trend of crude oil bioremediation using crushed room-dried *Azadirachta indica stem*.

4. CONCLUSION

A new model was developed to simulate the bioremediation of crude oil contaminated soils using *Azadirachta indica stem*. The experiment part of the study established the fact that 100g of blended room-dried *Azadirachta indica stem* caused the highest remediation in either sandy soil or loamy soil contaminated with 100ml of crude oil. Based on this observation, a biokinetic model was developed for both contaminated sandy soil and loamy soil treated with 100g blended room-dried *Azadirachta indica stem*.

These parameters, together with Monod's equation and material balance equation were then used to develop a model that simulates and predicts the process of crude oil bioremediation using *Azadirachta indica stem*. The TPH values obtained from the predictive model developed were similar to the TPH values gotten from the experiment. For SRD, TPH at 21 days was calculated to be 9085.47mg/kg which is similar to that gotten from experiment observed to be 9085.48mg/kg. The highest error was observed in day 7where calculated TPH was 23477.06mg/kg while the experimental value was 26865.7mg/kg. For LRD, TPH at 35 days was calculated to be 3707.81mg/kg which is similar to that gotten from experiment observed to be 3707.78mg/kg. The highest error was observed in day 7 where calculated TPH was 35707.07mg/kg while the experimental value was 37851.62mg/kg.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCE

1. Abbamondi, G., Tommonaro, G., Weyens N., Plant growth-promoting effects of rhizospheric and endophytic bacteria

associated with different tomato cultivars and new tomato hybrid. Chemical and Biological Technologies in Agriculture, 2016,(3), 1-6.
2. Abbassi, B.E., Shquirat, W.D., Kinetics of indigenous isolated bacteria used for ex-situ bioremediation of petroleum contaminated soil. Water, Air, & Soil Pollution, 2008,192, 221–226.
3. Abioye, O., Agamuthu, P., Abdulazziz, A., Biodegradation of Used Motor Oil in Soil Using OrganicWaste Amendments. Biotechnology Research International, 2012, 1-9.
4. Adekunle, A. A., Tobit, I.U., Assessing And Forecasting The Impact of Bioremediation Product Derived from Nigeria Local Raw Materials on Electrical Conductivity of Soils Contaminated With Petroleum Products. Journal of Applied Technology in Environmental Sanitation, 2013, 2(1), 57-66.
5. Agamuthu, P.T., Bioremediation of Hydrocarbon Contaminated Soil Using Selected Organics Wastes. International Symposium on Environmental Science and Technology, Procedia Environmental Sciences, 2013, 18, 694-702.
6. Agarry, S.A., Factorial Designs Application to Study Enhanced Bioremediation of Soil Artificially Contaminated with Weathered Bonny Light Crude Oil through Biostimulation and Bioaugmentation Strategy. Journal of Environmental Protection, 2012, 3, 748-759.
7. Al-Saleh, E., Akbar, A., Occurrence of Pseudomonas aeruginosa in Kuwaiti soil. . Chemosphere, 2015,120, 11-17.
8. Azadeh, K., Mahmoodzadeh, B., Novel mathematical models for prediction of microbial growth kinetics and contaminant degradation in bioremediation process. Journal Of Environmental Engineering and Landscape Management, 2016, 24(03), 157–164.
9. Basumatary, B., Bordoloi, S., Phytoremediation of Crude Oil-Contaminated Soil Using Cynodon dactylon (L.) Pers. Springer International Publishing Switzerland, 2016, 41-51.
10. Bhattacharjee, M., Mahanta, K., Synergistic Effect of Cyperus odoratus & PGPR Strain in Hydrocarbon Uptake From Oil Contaminated Soil. Biotechnology Industrial Journal, 2018, 14(3),165.
11. Chijioke-Osuji, C., Ibegbulam-Njoku P., Biodegradation of Crude Oil Polluted Soil by Co-Composting with Agricultural Wastes and Inorganic Fertilizer.Research Journal of Natural Sciences,2014, 4(6), 1-12.
12. Chukwuma, J.N., Performance Evaluation of Vernonia Galamensis and Vernonia Amygdalina Species in Bioremediation of Petroleum Contaminated Sandy Soils in the Niger Delta. International Journal of Engineering and Modern Technology,2018, 84 to 97.
13. Das Saha, P., Mathematical modeling of the reduction of safranin onto chemically modified rice husks in stirred tank reactor using response surface methodology and artificial neural network . Bioremediation Journal, 2013,17, 52–60.
14. Etim, O.E., Udosen, C., Identification of hydrocarbon degraders in a crude oil polluted vessel and possible growth inducing potential of azadirachta indica. Global Journal of Research on Medical Plants & Indigenous Medicine, 2013,492 to 498.
15. Ezenobi, N. O., Combined effect of temperature and pH on pseudomonas aeruginosa isolated from a cosmetic products . International Journal of Current Research, 2016, 37124 to 37130.
16. Fallgren, P.H., Jin, S., Zhang, R., Stahl, P.D., Empirical models estimating carbon dioxide accumulation in two petroleum hydrocarbon–contaminated soils. Bioremediation Journal, 2010, 14, 98–108.
17. Gaiero, J., Mccall, C., Inside the root microbiome: bacterial root endophytes and plant growth promotion. American Journal of Botany, 2013, 100(9), 1738-1750.
18. Geng, X., Boufadel, M.C., Wrenn, B., Mathematical modeling of the biodegradation of residual hydrocarbon in a variably-saturated sand column . Biodegradation,2013,24, 153–163.
19. Germaine, K.J., Keogh, E., Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. Federation of European Microbiological Societies Microbiology Letters, 2009, 296, 226-234.
20. Glick B., Todorovic, B., Promotion of plant growth by bacterial ACC deaminase. Critical Review in Plant Sciences, 2007, 227-242.
21. Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., He, Y.,Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium Bacillus sp. L14. Bioresource Technology, 2010, 101, 8599–8605.
22. Kaneez, F., Asma, I., Plant-Bacteria Synergism: An innovative approach for the bioremediation of crude oil-contaminated soil. Soil Environment, 2017, 36(2), 93-113.
23. Khan, S, Afzal M, Iqbal S, Khan Q.M., Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere, 2013, 4,1317–1332..
24. Koreiviene, J., Valciukas, R., Karosiene, J., & Baltrenas, P., Testing of Chlorella/Scenedesmus microalgae consortia for remediation of wastewater, CO_2 mitigation and algae biomass feasibility for lipid production. Journal of Environmental Engineering and Landscape Management, 2014, 22, 105–114.
25. Li, H.Y., Wei, D.Q., Endophytes and their role in phytoremediation. Fungal Divers, 2012, 1(54), 11-18.
26. Mohajeri, L., Aziz, H.A., Isa, M H., Zahed, M.A., Mohajeri, S., Effect of remediation strategy on crude oil biodegradation kinetics and half life times in shoreline sediment. International Journal of Marine Science and Engineering, 2013, 3, 99–104.

27. Neha, J., Swati S., Phytoextraction Potential of Neem (Azadirachta indica) for Cd detoxification. Climate Change and Environmental Sustainability, 2018, 6(2), 154-159.
28. Nikakhtari, H., Kumar, P., Nemati, M., Hill, G.A., Mass transfer and bioremediation of aromatics from NAPL in a baffled roller bioreactor. Process Safety and Environmental Protection, 2008, 86, 252–258.
29. Nwogu, T.A., Enhanced Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbons after Amendment with Capra aegagrus hircus (Goat) Manure . Biotechnology Research International,2015, 1-7.
30. Ofoegbu, R. M., Bioremediation of crude oil Contaminated Soil Using Organic and Inorganic Fertilizers. Petroleum and Environmental Biotechnology, 2015, 6(1), 1-6.
31. Sanni E.S, Emetere E., Mathematical Modelling of insitu-Bioremediation of Crude Oil Polluted Soil. Science and Engineering Applications, 2016,1(4), 27-32.
32. Santoyo G, Moreno-Hagielsie G., Plant growth-promoting bacterial endophytes. Microbiol Research, 2016, 183, 92-99.
33. Shallu S., Hardik, P., Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons. International Journal of Pure Applied Bioscience, 2014, 2320-7051.
34. Van Ginneken, L., Meers, E., Guisson, R., Ruttens, A., Elst, K., Tack, F.M., Dejonghe, W., Phytoremediation for heavy metal contaminated soils combined with bioenergy production. Journal of Environmental Engineering and Landscape Management, 2007, 15, 227–236.
35. Vangronsveld J., Herzig R., Phytoremediation of contaminated soils and groundwater: lessons from the field. Evironmental Science and Pollution Research, 2009, 16, 765-794.
36. Wang, J., Zhang Z., Phytoremediation of petroleum polluted soil. Petroleum Science, 2008, 5, 167-171.
37. Weyens, N, Targhavi, S., Bacteria associated with oak and ash on a TCE-contaminated site: characterization of isolates with potential to avoid evapotranspiration.Environmental Science and Pollution Research, 2009, 16, 830-843.
38. Yaohui, X., Maang L., Bioremediation of Crude Oil-contaminated Soil: Comparison of Different Biostimulation and Bioaugmentation Treatments. Journal of Hazardous Materials, 2010, 395-401.
39. Yousaf, S, Afzal, M., Hydrocarbon degradation, plant colonization and gene expression of alkane degradation genes by endophytic Enterobacter ludwigii strains. Environmental Pollution, 2011, 10, 2675-2683.